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TOXICITY AND FATE OF NICKEL-COATED GRAPHITE IN A MARINE ENVIRONMENT

M.V. Haley
C.W. Kurnas

RESEARCH AND TECHNOLOGY DIRECTORATE

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The toxicity of nickel-coated graphite fibers was examined using the marine amphipod *Ampelisca abdita.* Fibers were mixed into the top 1 cm of sediment at concentration from 10 to 50 mg/L. The 10-day EC_{50} to amphipods was 40 mg/L. Fate studies (21 days) were conducted in salt water with and without sediment. Fate studies were also conducted with the fibers ground to powder. Ground fibers allowed a higher concentration of nickel to dissolve into the water column. However, when mixed into sediment, the concentration of dissolved nickel is significantly less.

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PREFACE

The work described in this report was authorized under Sales Order No. 1J2L. This work was started in June 1994 and completed in December 1994.

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TOXICITY AND FATE OF NICKEL-COATED GRAPHITE
IN A MARINE ENVIRONMENT

1. INTRODUCTION

There is a general interest to the military services in various candidate obscurant materials. Knowing the effects of these materials on the environment is critical for their acceptance. One material of concern is nickel-coated graphite (NCG), for which aquatic toxicity data are lacking.

Most open literature reports examine the toxicity and fate of metal salts, overlooking the fact that metals can be introduced into the environment as particles or filings. The possibility exists that the NCG may be released into aquatic ecosystems through military maneuvers and field testing/training. Earlier research investigated the acute effects of nickel coated graphite on pelagic organisms (daphnia and fish) and did not address the possible effects on benthic organisms.[1] Repeated exposures to aquatic ecosystems may result in the accumulation of enough nickel in bottom sediments to cause detrimental effects on benthic organisms.

Recent studies conducted at the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), M.V. Haley et. al.,[1,2] comparing iron-coated glass, aluminum-coated glass, and NCG fibers, have shown NCG to be toxic to *Daphnia magna* and nontoxic to fathead and sheepshead minnows. Also, fate studies have shown that the nickel coating dissolves at various rates depending on water hardness and salinity.

The research presented in this paper focuses on the effects of NCG on the benthic invertebrate *Ampelisca abdita*, a marine amphipod. This organism lives within the top 1 cm of sediment, concealed in a detritus tube that is open to the sediment surface. Due to this organism's life style and habitat, it could potentially receive an extremely high exposure to fibers deposited in the bottom sediments. Fate studies were also conducted on NCG after being deposited in sediment. The overlaying water was monitored for dissolved nickel and compared to previously conducted fate studies without sediment.[1]

2. FATE DETERMINATION

To provide more uniform distribution of the NCG fibers when added to the test chamber, fibers were ground to a powder using a high speed micro grinding mill by Brinkmann (Brinkmann Instruments, Westbury, NY). The fibers were placed into the grinding chamber in 0.25 g batches and ground until there were no noticeable whole intact fibers. After grinding, the powder was stored under desiccation until needed.

Marine sediment (200 mL) from Narraganset, RI, was placed in 1 L jars with screw top lids. The containers were lightly tapped on the counter to force the air pockets out of the sediment. Ground NCG was mixed into the top 1 cm of sediment to yield concentrations of 50, 100, 150, 200, 250, and 1000 mg/L (concentration based on the entire volume of the container). Natural sea water (600 mL, also from Narraganset, RI) was added to the container using a turbulence reducer to prevent sediment suspension and release of the nickel powder into the water column. Positive controls of 1000 mg/L without sediment were used to compare the effects of sediment on the concentration of

dissolved nickel present in the overlying water column. There were two replicates prepared for each treatment group and controls. The containers were allowed to set at room temperature (20 ± 2 °C), with constant aeration. Two 10-mL samples were removed from the vessels on day 1, 2, 3, 7, 14, and 21. The samples were filtered through a 0.45 μ filter and analyzed for nickel using atomic absorption (AA) spectroscopy (for instrument settings see Table 1).

Table 1. Perkin Elmer 460 Instrument Settings for Nickel Analysis

Wave Length	232 nm
Slit Size	0.2 mm
Flame	Air/Acetylene
Lamp Power	30 ma
Signal	AA
Integration Time	2.0 s

Results were plotted against a standard curve and subjected to regression analysis to determine the concentration of dissolved nickel in solution.

Dissolved nickel concentrations were subjected to analysis of variance (ANOVA) and Dunnett's Multiple Comparison Test to determine significant differences among treatment groups.

3. SEDIMENT TOXICITY ASSAY

The sediment toxicity assay exposed *A. abdita,* to ground nickel-coated graphite. The nickel-graphite fibers were ground to a powder to yield a more uniform distribution under experimental conditions. Also, the ground material has a much higher surface area which should yield maximum dissolved nickel concentrations and represent a worst case scenario.

East Coast Amphipods, Incorporated (Kingston, RI), collected amphipods from sites located in Narraganset, RI.[3] The organisms, native sea water, and sediment were shipped via overnight freight. The organisms were placed in 10-gal glass aquarium with 4 cm of sediment and allowed to acclimate to test conditions for 48 hr.

The test chambers consisted of l-L glass jars from Scientific Specialties Services Incorporated (Randallstown, MD).[4] Sediment was passed through a 2 mm stainless steel sieve to remove large debris and possible predatory organisms. After the sediment was sieved, enough was placed into the test chambers to provide approximately 4 cm of depth (200 mL). Ground nickel graphite was added to the surface of the sediment and then mixed into the top 1 cm using disposable plastic pipettes. Sea water was added to the test chamber, using a turbulence reducer to prevent the disturbance of the sediment. The test chambers were covered and gently aerated with a pipette, inserted through the lid, to

approximately 1-2 cm above the sediment. Air flow was adjusted so re-suspension of sediment would not occur. After 24 hr of aeration, the test chambers were randomly placed in a temperature controlled room (20 ± 2°C), with a light/dark cycle of 16/8 hr.

Amphipods were removed from the acclimation aquarium by collecting the bottom sediments and sifting the mud and debris through a 0.5 mm stainless steel sieve. The animals were placed in shallow trays containing fresh sea water. Immature amphipods of uniform size were transferred sequentially, via pipette, to sample cups (20 animals each) to reduce bias in animal choice.

When the amphipods were added to the test chambers, aeration was stopped and the sample cups containing the amphipods were poured through a 0.5 mm stainless steel screen to remove the water. The amphipods were recounted and rinsed off the screen into the test chambers. Any floating amphipods were gently pushed under the water surface with a pipette. The animals were allowed 1 hr to burrow into the mud. Any amphipods not submerged into the sediment were removed and replaced.

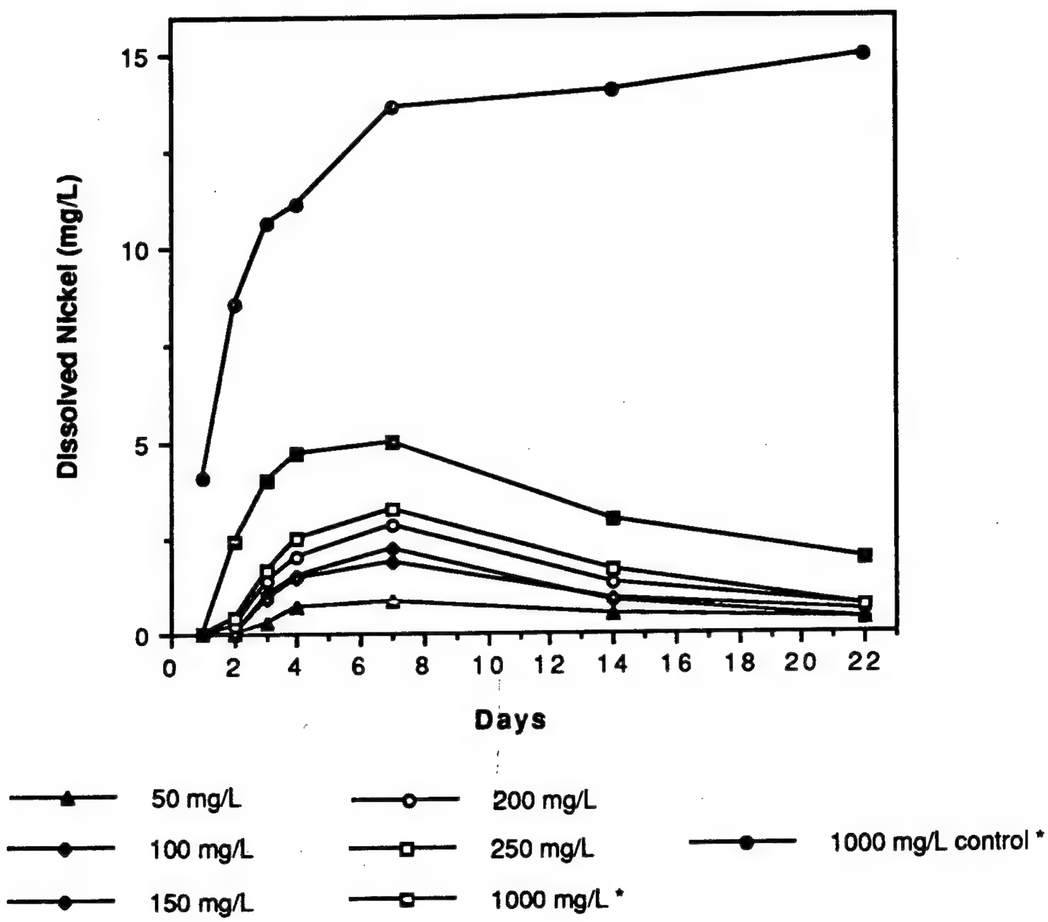
Daily observations were conducted. Any floating animals (live) were noted and submerged. Any motionless animals were removed and examined under a dissecting microscope. If any sign of life (neuromuscular twitching) was observed, they were returned to the test chamber and gently submerged into the sediment.

At the conclusion of testing (10 days), water samples were removed for nickel analyses and mortality determined. To determine mortality, the contents of the test chambers were rinsed individually through a 0.5 mm sieve, using sea water to remove mud and expose the amphipods for counting. If 16 out of 20 animals were not found, including any dead animals reported during the test, the sample was examined by another technician for quality control purposes. All animals not found were considered dead. The EC_{50} (concentration that effected 50% of the organisms) determinations were made using Probit analysis and confirmed using regression analyses.[3]

4. RESULTS

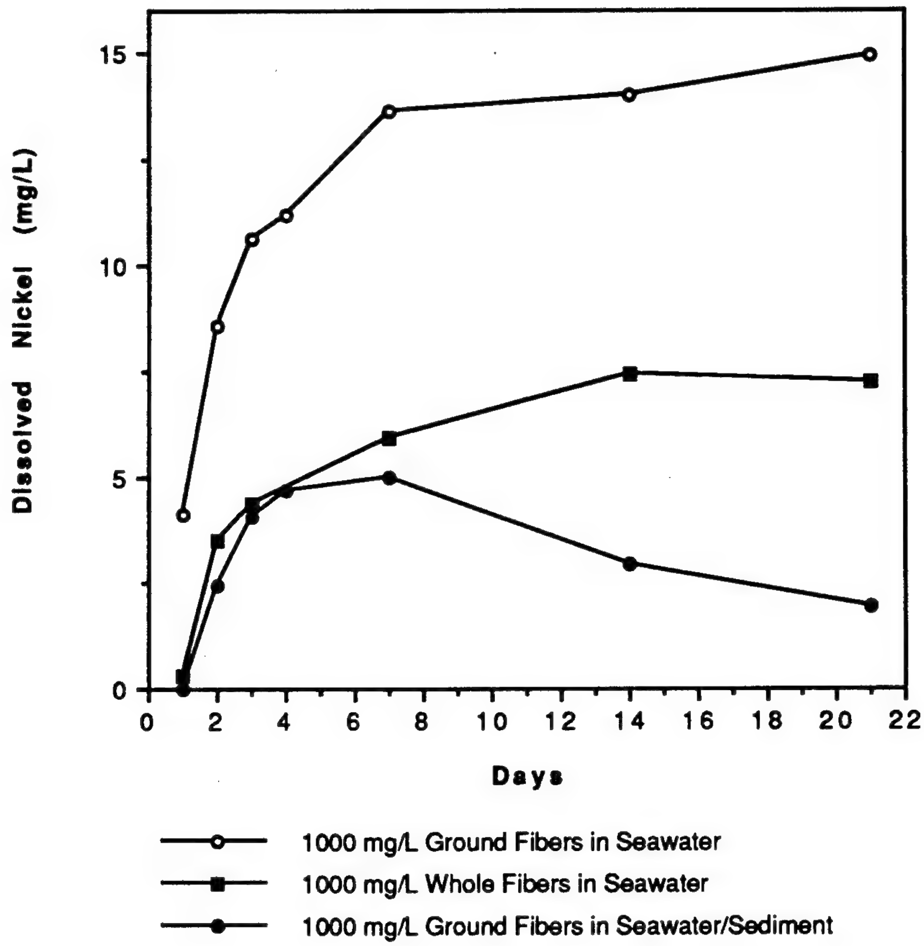
Dissolved nickel concentrations, during the fate study, increased rapidly and reached equilibrium in approximately 7 days (Figure 1). The dissolved nickel concentration for the 1000 mg/L treatment group reached a maximum of 5 mg/L. After 7 days, all the treatment groups containing sediment showed a decrease in dissolved nickel. The concentration of dissolved nickel in the 1000 mg/L control (no sediment) was approximately four times higher than that of the 1000 mg/L treatment group with sediment (significant difference at $p \leq 0.05$). The concentration of dissolved nickel from whole fibers in sea water (Figure 2) was similar to the concentration of nickel produced from the ground fibers mixed into the sediment. However, after 7 days the nickel concentration in the sediment group dropped. For comparison purposes, test results from fate studies conducted by Haley et. al.[1] using whole fibers are included in Figure 2.

Water samples from the sediment toxicity assay were taken at the conclusion of the test and analyzed for dissolved nickel. The dissolved nickel in the 50 mg/L toxicity assay treatment group was higher (3.1 mg/L, Figure 3) than the nickel concentration in the 50 mg/L fate study treatment group of the fate studies in sediment (0.84 mg/L, Figure 1).



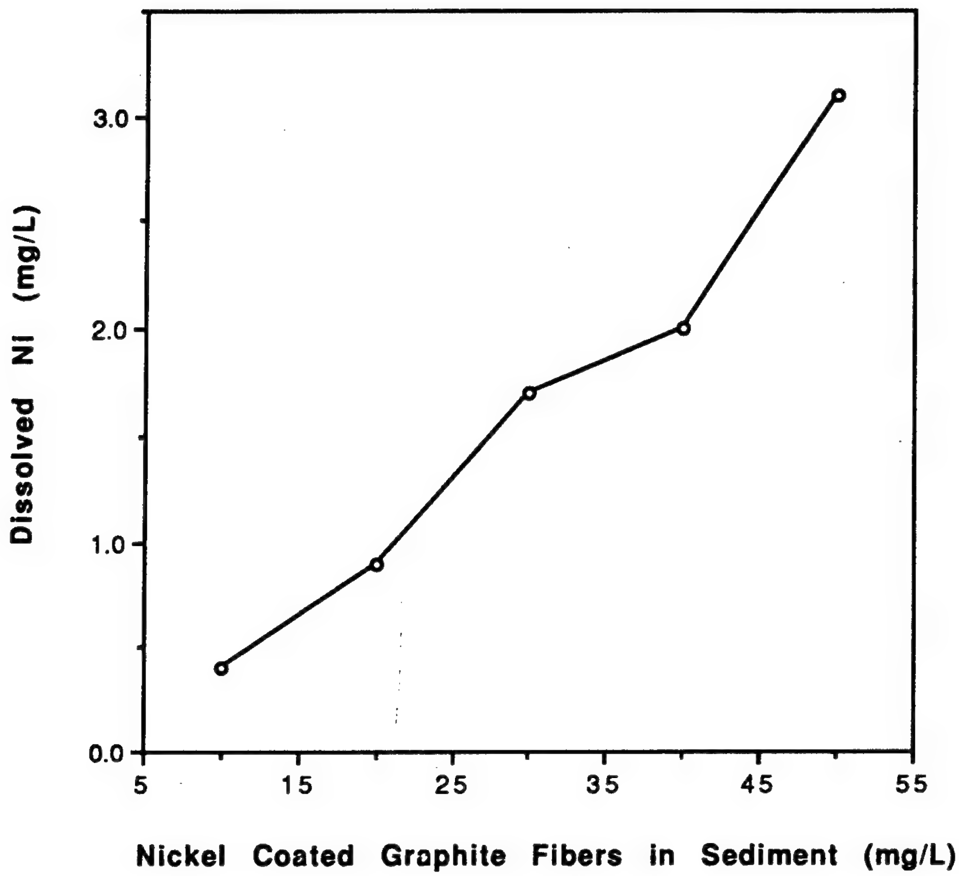
Note: The testing pararmeters during the fate studies were set to simulate the same conditions under which the toxicity assays were conducted. The 1000 mg/L control (no sediment) had dissolved nickel concentrations significantly higher ($p < 0.05$) than the treatment groups having sediment.

Figure 1. Nickel-Coated Graphite Fibers in Marine Sediment



Note: The samples of ground fibers in sea water without sediment had significantly more dissolved nickel in solution than the samples of ground fibers in sea water with sediment.

Figure 2. Nickel-Coated Graphite Fate Comparison



Note: Water samples were taken at day 10 and analyzed for dissolved nickel. Dissolved nickel in the 50 mg/L treatment group was approximately four times higher than the same treatment group during the fate studies.

Figure 3. Dissolved Nickel in the Water Column During Toxicity Assays

The 10-day EC_{50} (the concentration that which 50% of the organisms survived) for NCG to amphipods was 40 mg/L. Table 2 below compares the toxicity of several other metal materials to nickel coated graphite. Using the Chemical Scoring System for Hazard and Exposure Identification,[4] nickel coated graphite received a score of 4 (moderately toxic to marine amphipods). The scoring criteria is based on a scale from 1 to 9, where 9 is the most toxic. Comparative toxicity scores for other metal coated fibers are listed in Table 2.

Table 2. Toxicity of Selected Particulate Materials

TEST MATERIAL	TEST SPECIES**	EC_{50} (mg/L)	EPA* Ranking
Brass Flakes	*Daphnia magna*	0.021	9
	Ankistrodesmus falcatus	0.242	9
	Selenastrum capricornutum	0.087	9
Silica	*Daphnia magna*	>1000.0	0
Titanium Dioxide	*Daphnia magna*	>1000.0	0
Teflon	*Daphnia magna*	>1000.0	0
Stainless Steel	*Daphnia magna*	>5000.0	0
	Fathead Minnow	>1000.0	0
	Ampelisca abdita	>1000.0	0
Iron-Coated Glass Fibers	*Daphnia magna*	>1000.0	0
	Sheepshead Minnow	>1000.0	0
	Fathead Minnow	>1000.0	0
	Mysid Shrimp	>1000.0	0
	Ampelisca abdita	>1000.0	0
Aluminum-Coated Glass Fibers	*Daphnia magna*	>1000.0	0
	Sheepshead Minnow	>1000.0	0
	Fathead Minnow	>1000.0	0
	Mysid Shrimp	>1000.0	0
	Ampelisca abdita	>1000.0	0
Nickel-Coated Graphite	*Daphnia magna*	>1000.0	0
	Sheepshead minnow	>1000.0	0
	Fathead minnow	>1000.0	0
	Ampelisca abdita	40.0	4

*Toxicity ranking based on the EPA chemical scoring system for hazard and exposure identification. Scoring is based on a scale of 0-9, 9 being the most toxic. The authors have rated the scale with the following potency levels: 0-3 (not toxic to low toxicity), 4-5 (moderate toxicity), and 6-9 (high toxicity).

**All *D. Magna* and *A. abdita* EC_{50} results are 48 hr in duration.
All *A. falcatus, S. capricornutum,* and Sheepshead minnow EC_{50} results are 96 hr in duration.

5. DISCUSSION

The marine amphipod *Ampelisca abdita,* inhabit the upper 1 cm of sediment, only venturing out of their tubes at night to forage on the bottom. Most of the amphipods life cycle is spent within the upper 1 cm of the water sediment interface.

In these toxicity studies, the NCG fibers were mixed into the top 1 cm of the sediment; therefore, the amphipods were in direct contact with the fibers in a closed system. The concentrations used in these studies may not be realistic environmental exposures. Environmental conditions such as wind, rain water run off, and topography all contribute to how a smoke plume dissipates. The Gaussian plume dispersion model[5] is used to describe the deposition of a smoke cloud. However, it does not model the dispersion of smoke particles in water. This model would only yield information on the concentration that was deposited on the water surface. Therefore, this model would not be good for predicting the concentrations of NCG deposition in aquatic ecosystems.

The concentrations of dissolved nickel from fate studies were compared to the concentrations of dissolved nickel produced in the toxicity assays that contained sediment. The amount of dissolved nickel in the toxicity studies at 50 mg/L treatment group (comparison to lower treatment groups are not available because the lowest fate study concentration was 50 mg/L) was approximately 4 times greater than dissolved nickel levels in the fate studies (also containing sediment). The difference in dissolved nickel is speculated to be due to the activity of the organisms in the sediment.

The results clearly show that there is a significant inhibitory effect on the concentration of dissolved nickel in the water column when mixed in the sediment. For example, a comparison was made between 1000 mg/L nickel-coated graphite placed in sea water and 1000 mg/L NCG mixed into the top 1 cm of sediment. At day 7 (day seven levels were used because the dissolved nickel peaked in the treatment groups containing sediment), the concentration of dissolved nickel in the treatment group with sea water was approximately 40% higher than the treatment group with sediment. There are two possible reasons for the difference in dissolved nickel. The sediment covering the nickel created a concentration gradient that limited further dissolution and only the nickel at the very top of the sediment dissolved in to the overlaying water. In the presence of sediment with high organic content, chelating materials in its matrix attached to the nickel to form complexes that prevent as much nickel from leaching into the overlaying water.[6,7] Whichever the case may be, it has been clearly demonstrated that when NCG is deposited in the bottom sediments, the dissolved nickel concentrations in the overlaying water column were significantly less than the nickel concentrations in the water column with out sediment. The organic content and type (sand, clay, mud) of bottom sediment may play a large role in the amount of dissolved nickel available to organisms, therefore effecting the toxicity of NCG to aquatic organisms.

The fate and toxicity of metals in the aquatic environment are extremely important issues because metals accumulate in the tissues of aquatic organisms.[8,9] Nickel, however, has not been reported in the open literature to bioaccumulate in significant amounts (aquatic species) and is not considered to be an immediate human health factor.

Studies conducted by Haley et al. have shown that NCG fibers were not toxic to Fathead minnows, Sheepshead minnows, and *Daphnia magna* up to 1000 mg NCG fibers/L.[2] When daphnia were exposed to concentrations of dissolved nickel the

concentration that immobilized 50% of the daphnia (EC50) was 1 mg/L. However, 1000 mg NCG fibers/L did not yield enough dissolved nickel to immobilize daphnia. These results show that when nickel is bound and unavailable to aquatic organisms (still attached to the fiber), the toxicity results could be orders of magnitude different. As suggested by Cataldo et. al.,[5] the toxic effects due to metal partitioning need to be investigated in further detail to address the impact that NCG has on an aquatic ecosystem.

The study presented in this paper, is short term and does not address long term effects such as life cycle, growth rate, and reproduction. If prolonged use of nickel-coated graphite is expected, it is recommended to conduct studies to determine the long term effects of nickel-coated graphite on the environment.

6. CONCLUSIONS

Nickel-coated graphite fibers were moderately toxic to the marine amphipod, *Ampelisca abdita.* The presence of sediment has a significant effect on the concentration of dissolved nickel in the water column. Modeling is recommended to predict the concentration of obscurant fibers that would realistically be deposited into bottom sediment. Grinding the fibers to a powder produces twice the concentration of dissolved nickel when compared to whole fibers. Therefore, the toxicity results presented in this paper represent worst case scenario. If long-term continued exposure is expected, long-term chronic effects on the surrounding ecosystem should be determined.

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